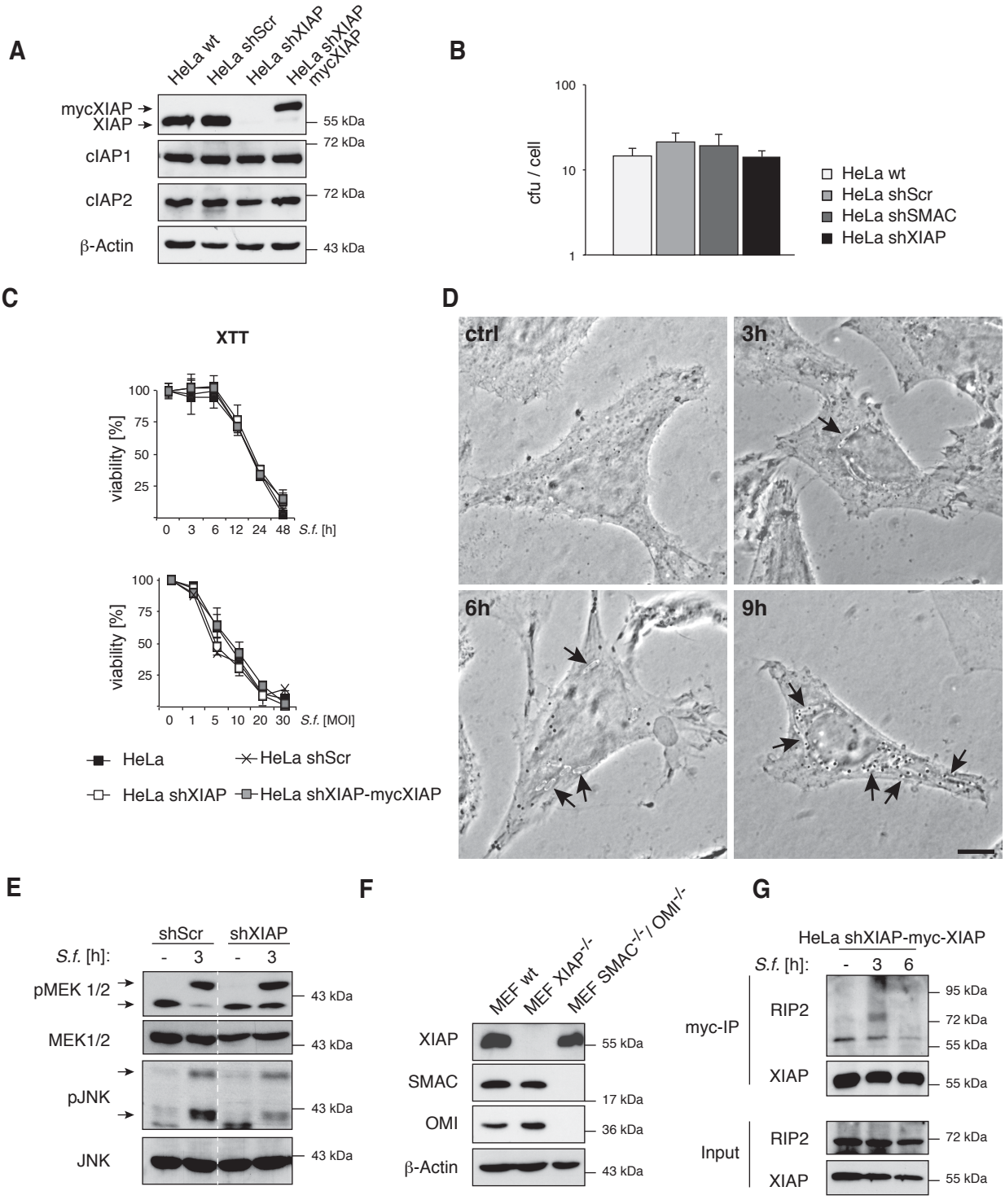
**SUPPLEMENTARY INFORMATION**

**Fig S1**




**Supplementary Figure 1: XIAP is required for *Shigella*-induced NF-κB activation**

(A) Expression levels of XIAP and cIAP1/2 in HeLa wt, HeLa shScr, HeLa shXIAP and HeLa shXIAP-mycXIAP were analyzed by Western blotting.

(B) HeLa wt, HeLa shScr, HeLa shSMAC and HeLa shXIAP cells were infected with *Shigella* M90T (MOI 30) and a gentamycin protection assay was performed. Colony forming units were counted and calculated bacteria per cell are presented. Data are presented as mean ± SD (n=4).

(C) HeLa wt, HeLa shScr, HeLa shXIAP and HeLa shXIAP-mycXIAP cells were infected with *Shigella* M90T with MOI 30 (upper panel) or the indicated MOI (lower panel). Cell viability was determined at the indicated time points *p.i.* by trypan blue exclusion. Data are presented as mean ± SD (n=3).

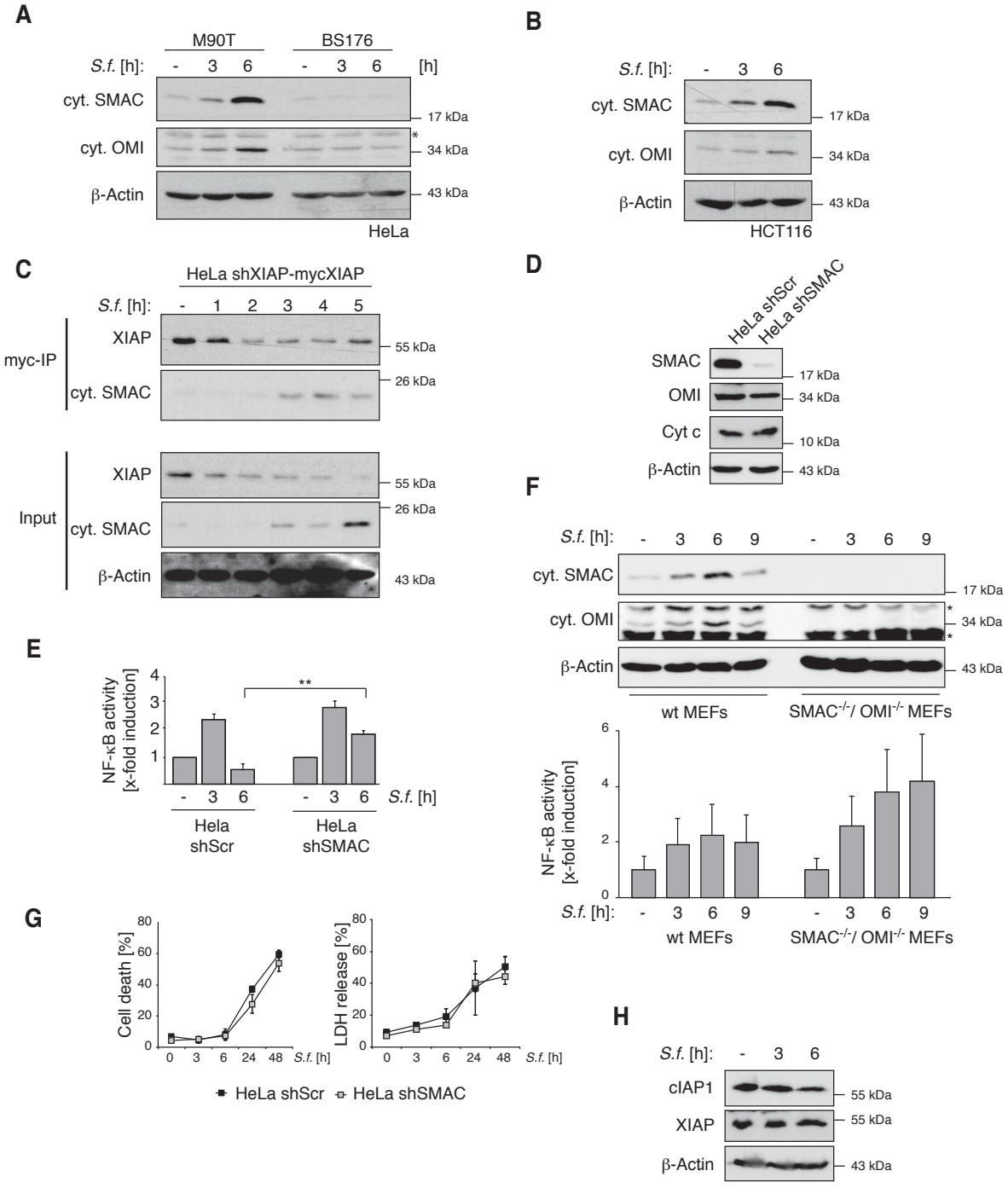
(D) HeLa wt cells were left untreated (ctrl) or were infected with *Shigella* infected with *Shigella* M90T (MOI 30). At the indicated time points cells were fixed and monitored by bright field microscopy. Arrows point at intracellular *Shigella.* (scale bar = 10 µm)

(E) HeLa shScr and HeLa shXIAP were left untreated (-) or were infected with *Shigella* M90T (MOI 30). Whole cell extracts were analyzed by Western blotting.

(F) Expression levels of the indicated proteins in MEFs derived from wt, $XIAP^{-/-}$ and $SMAC^{-/-}/OMI^{-/-}$ mice analyzed by Western blotting.

(G) HeLa shXIAP-mycXIAP cells were left untreated (-) or were infected with *Shigella* M90T (MOI 30). A myc-tag immunoprecipitation (IP) was performed with cytosolic extracts at the indicated time points *p.i.*. Input and IP were analyzed by Western blotting.

**Fig S2**




**Supplementary Figure 2: *Shigella* induces the release of mitochondrial SMAC and inhibits XIAP-mediated inflammatory signaling**

(A) HeLa wt cells were left untreated (-) or were infected with invasive *Shigella* M90T or the non-invasive control strain BS176 (MOI 30). Cytosolic extracts were analyzed by Western blotting at the indicated time points *p.i.*. (* marks unspecific bands).

(B) HCT116 cells were left untreated (-) or were infected with *Shigella* M90T (MOI 30). Cytosolic extracts were analyzed by Western blotting at the indicated time points *p.i.*.

(C) HeLa shXIAP-mycXIAP cells were left untreated (-) or were infected with *Shigella* M90T (MOI 30). A myc-tag immunoprecipitation (IP) was performed with cytosolic extracts at the indicated time points *p.i.*. Input and IP were analyzed by Western blotting.

(D) Expression levels of the indicated proteins in HeLa shScr and HeLa shSMAC, analyzed by Western blotting.

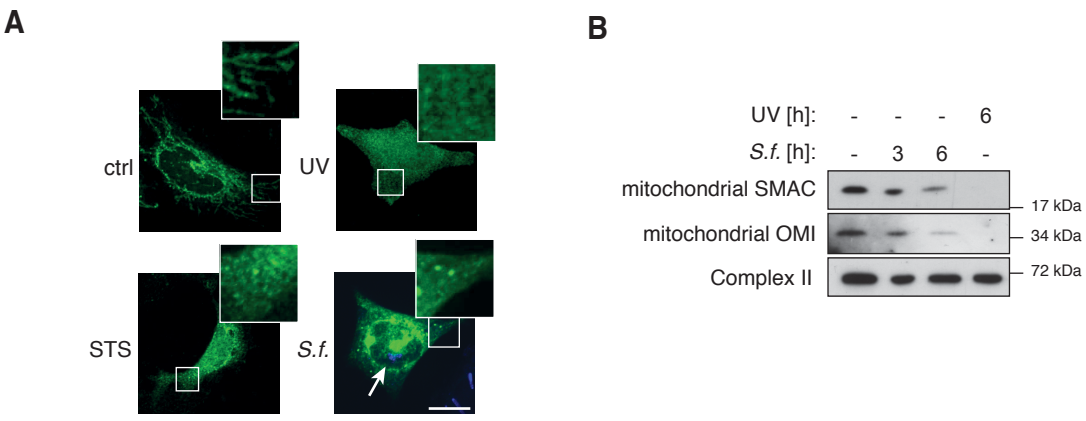
(E) Densitometric quantification of NF-κB DNA binding activity of at least three independent experiments shown in Figure 2B. $^{**}p < 0.01$.

(F) MEFs isolated from wt or $SMAC^{-/-}/OMI^{-/-}$ mice were left untreated (-) or were infected with *Shigella* M90T (MOI 50). Cytosolic fractions were analyzed by Western blotting at the indicated time points *p.i.* (upper panel, * marks unspecific bands). NF-κB activity was analyzed by ELISA at the indicated time points *p.i.* (lower panel). Data are presented as mean ± SEM (n=4).

(G) HeLa shScr and HeLa shSMAC cells were infected with *Shigella* M90T (MOI 30). Cell death (left panel) and LDH release (right panel) were determined by trypan blue exclusion and the Cytotoxicity Detection Kit, respectively, at the indicated time points *p.i.*. Data are presented as mean ± SD (n=3).

(H) HeLa wt cells were left untreated (-) or were infected with *Shigella* M90T (MOI 30). Cytosolic extracts were analyzed by Western blotting at the indicated time points *p.i.*.

**Fig S3**

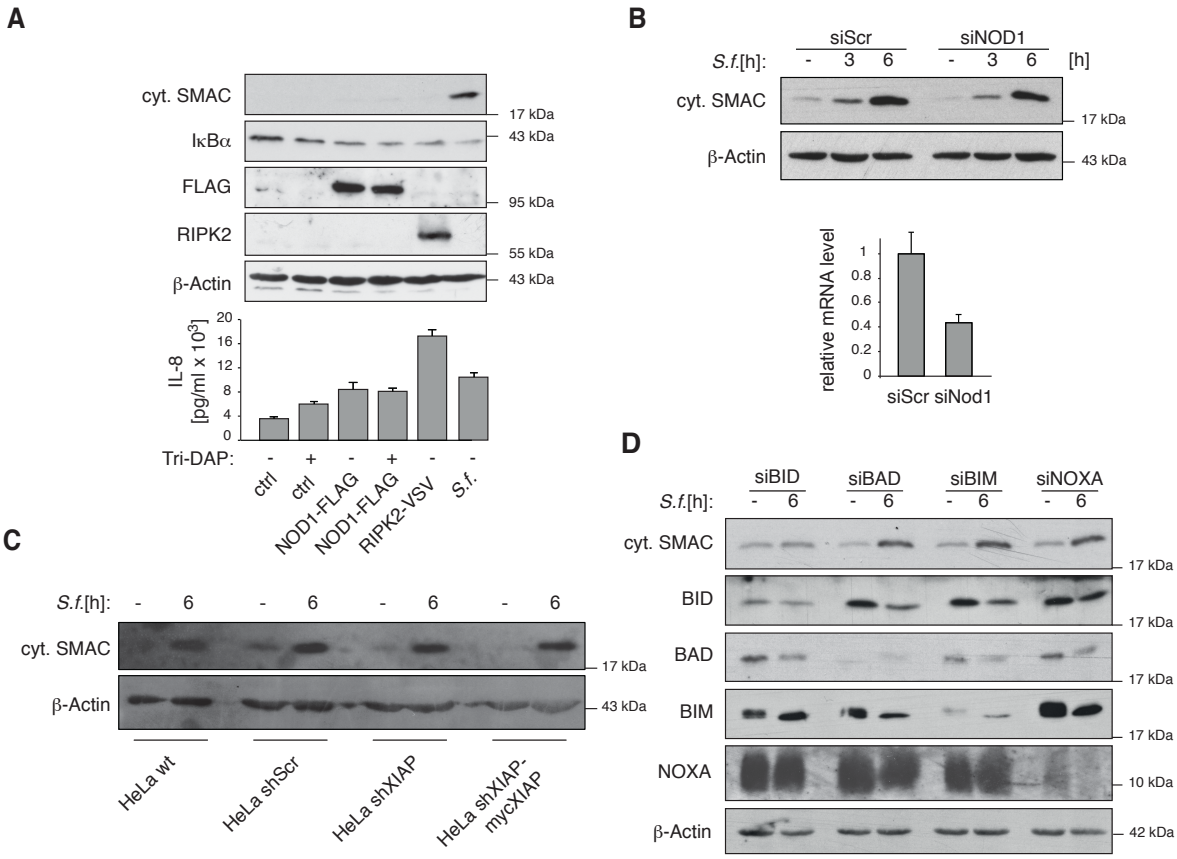



**Supplementary Figure 3: Intracellular *Shigella* induces the release of mitochondrial SMAC without inducing mitochondrial damage**

(A) HeLa wt cells were left untreated (ctrl), treated with UV-light (10 mJ/m$^2$), STS (0.5 $\mu$M) or were infected with *Shigella* M90T (MOI 10). After 4 h cells were fixed and SMAC (green) and *Shigella* (blue) were stained blue by immunofluorescence. (Arrow points to intracellular *Shigella*).

(B) HeLa wt were left untreated (-), treated with UV light (10 mJ/m$^2$) or were infected with *Shigella* M90T (MOI 30). Mitochondria were isolated at the indicated time points *p.i.* and analyzed by Western blotting.

**Fig S4**




**Supplementary Figure 4**: **BID induces the mitochondrial release of SMAC in *Shigella*–infected cells**

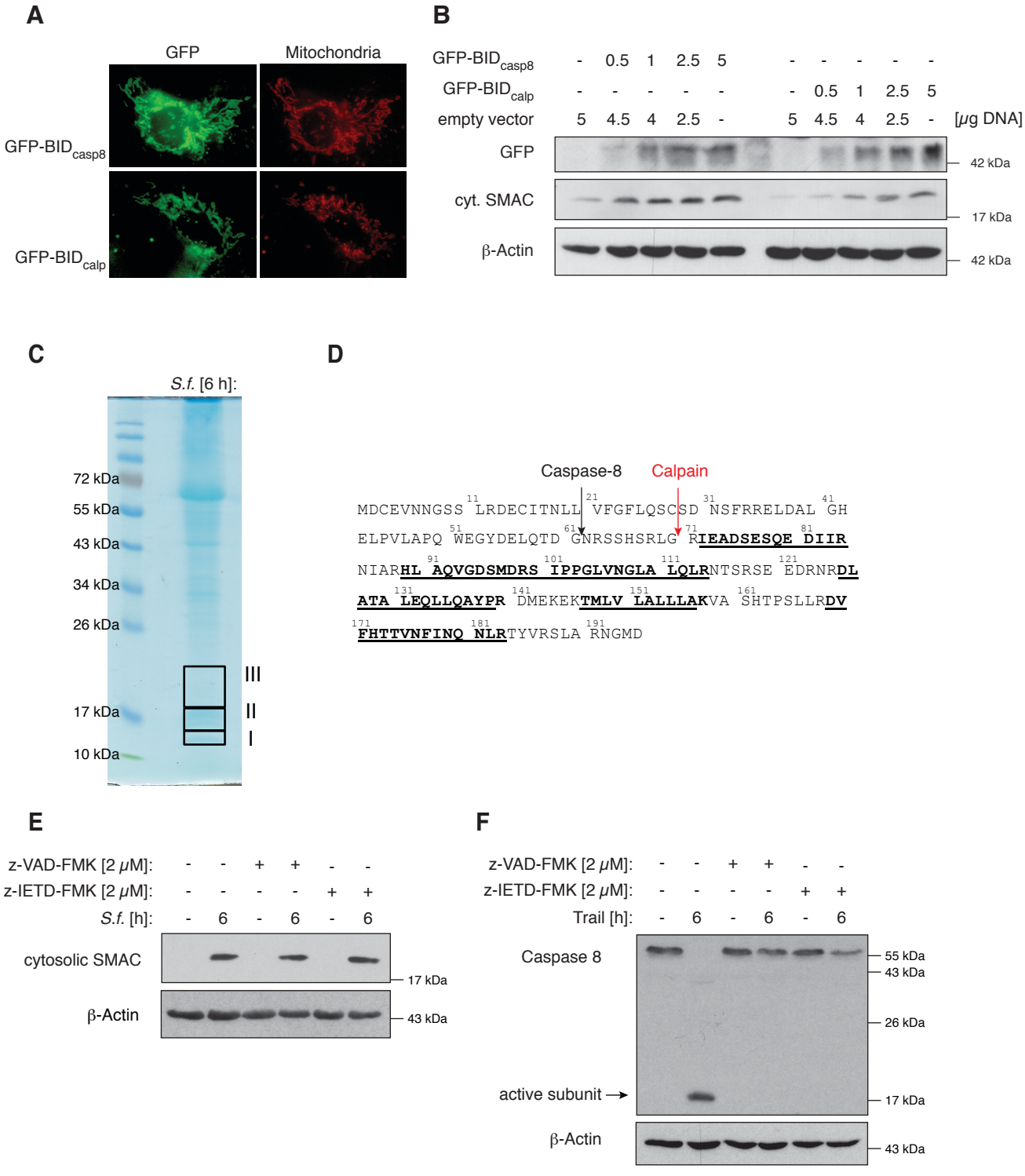
(A) HeLa wt cells were left untreated (ctrl) or transfected with expression vectors encoding NOD1-Flag or RIPK2-VSV. After 24 h, cells were stimulated with Tri-DAP (10 μg/ml) (+) or infected with *Shigella* M90T (MOI 30). Cytosolic fractions were analyzed by Western blotting 6 h *p.i.* (upper panel). IL-8 secretion was monitored by ELISA in supernatants of three independent experiments 6 h *p.i.* (lower panel). Data are presented as mean ± SEM (n=3).

(B) HeLa wt cells were transiently transfected with control siRNA (siScr) or a specific siRNA for NOD1. After 48 h cells were infected with *Shigella* M90T (MOI 30). At the indicated time points *p.i.* cytosolic extracts were analyzed by Western blotting (upper panel). NOD1 knock-down was controlled by RT-PCR (lower panel).

(C) HeLa wt, HeLa shScr, HeLa shXIAP and HeLa shXIAP-mycXIAP cells were left untreated (-) or were infected with *Shigella* M90T (MOI 30). Cytosolic extracts were analyzed by Western blotting 6 h *p.i.*.

(D) HeLa wt cells were transiently transfected with specific siRNAs for BID, BAD, BIM or NOXA. After 48 h cells were left untreated (-) or were infected with *Shigella* M90T (MOI 30). Cytosolic extracts were analyzed by Western blotting 6 h *p.i.*.

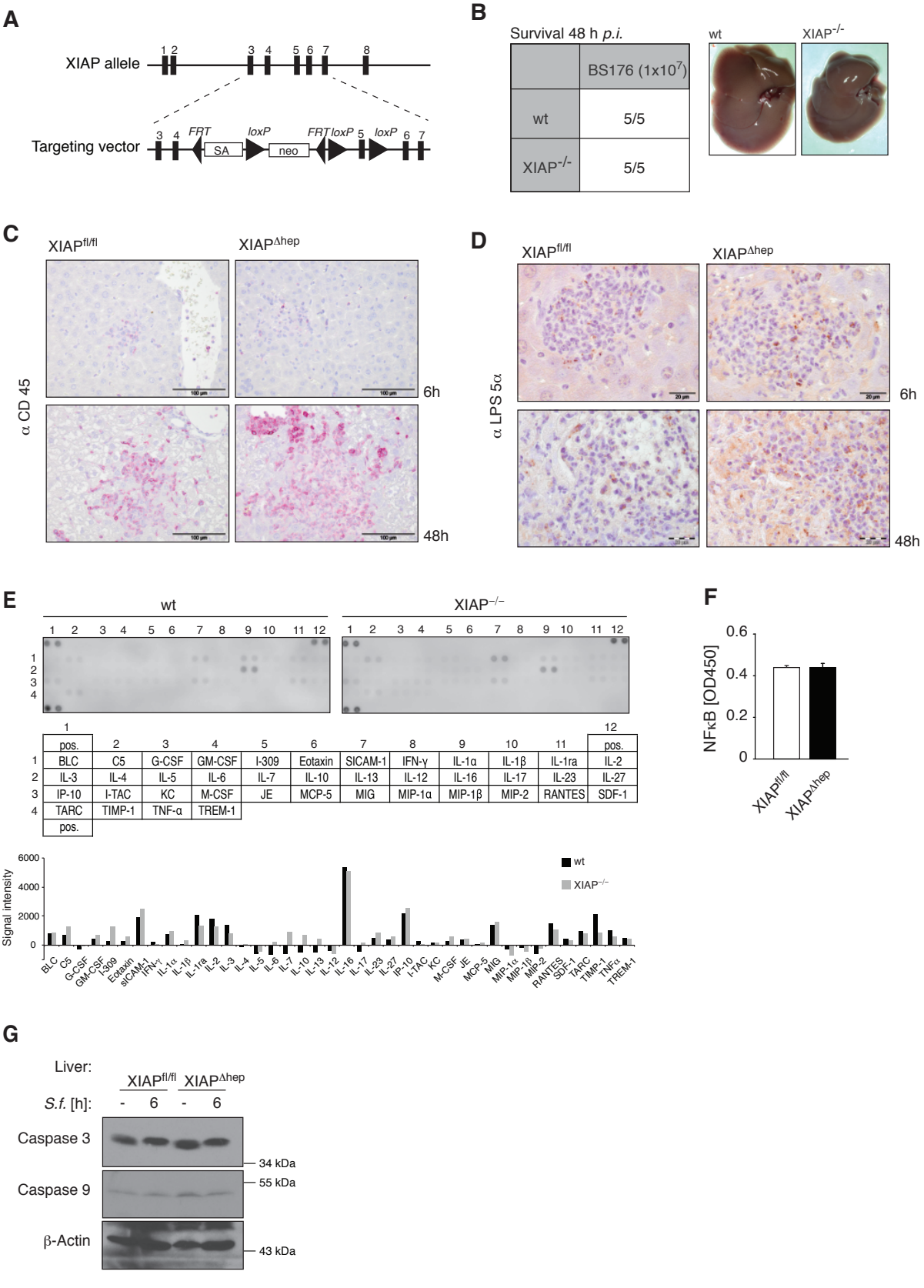
**Fig S5**



**Supplementary Figure S5**: **BID is cleaved by Calpain after infection with *Shigella***

(A) HeLa wt cells were transiently transfected with GFP-$BID_{casp8}$ or GFP-$BID_{calp}$ in combination with pKindling-Red mito and analyzed by fluorescence microscopy after 24 h.
(B) HeLa wt cells were transiently transfected with the indicated amounts of expression vectors encoding GFP-$BID_{casp8}$, GFP-$BID_{calp}$ or empty GFP-vector. After 24 h mitochondria were analyzed for GFP expression and cytosolic extracts were analyzed for released SMAC by Western blotting.
(C) HeLa wt cells were infected with *Shigella* M90T (MOI 30). Mitochondrial fractions were separated by SDS-PAGE and stained with Coomassie. Three bands were cut out (I, II, III) and analyzed by mass spectrometry.
(D) Schematic sequence coverage of BID peptides identified by mass spectrometry. Found BID sequences are marked in bold. Caspase 8 and Calpeptin cleavage sites are indicated.
(E) HeLa wt cells were left untreated (-) or were infected with *Shigella* M90T (MOI 30) in combination with z-VAD-FMK or z-IETD-FMK. Cytosolic extracts were analyzed by Western blotting 6 h *p.i.*.
(F) HeLa wt cells were left untreated (-) or were treated with TRAIL (50 ng/ml) in combination with z-VAD-FMK or z-IETD-FMK. Cytosolic extracts were analyzed by Western blotting 6 h *p.i.*.

**Fig S6**


A
XIAP allele
1 2 3 4 5 6 7 8
Targeting vector
3 4 FRT SA loxP neo FRT loxP 5 loxP 6 7
B
Survival 48 h p.i.
BS176 (1x10^7)
wt 5/5
XIAP-/- 5/5
wt
XIAP-/-
C
XIAPfl/fl
XIAPΔhep
α CD 45
6h
48h
D
XIAPfl/fl
XIAPΔhep
α LPS 5α
6h
48h
E
wt
XIAP−/−
1 2 3 4 5 6 7 8 9 10 11 12
1 pos. BLC C5 G-CSF GM-CSF I-309 Eotaxin SICAM-1 IFN-γ IL-1α IL-1β IL-1ra 12 pos. IL-2
2 IL-3 IL-4 IL-5 IL-6 IL-7 IL-10 IL-13 IL-12 IL-16 IL-17 IL-23 IL-27
3 IP-10 I-TAC KC M-CSF JE MCP-5 MIG MIP-1α MIP-1β MIP-2 RANTES SDF-1
4 TARC TIMP-1 TNF-α TREM-1 pos.
Signal intensity
6000
4000
2000
0
wt
XIAP−/−
BLC C5 G-CSF GM-CSF I-309 Eotaxin sICAM-1 IFN-γ IL-1α IL-1β IL-1ra IL-2 IL-3 IL-4 IL-5 IL-6 IL-7 IL-10 IL-13 IL-12 IL-16 IL-17 IL-23 IL-27 IP-10 I-TAC KC M-CSF JE MCP-5 MIG MIP-1α MIP-1β MIP-2 RANTES SDF-1 TARC TIMP-1 TNFα TREM-1
F
NFκB [OD450]
0.6
0.4
0.2
0
XIAPfl/fl
XIAPΔhep
G
Liver:
XIAPfl/fl XIAPΔhep
S.f. [h]: - 6 - 6
Caspase 3
34 kDa
Caspase 9
55 kDa
β-Actin
43 kDa

**Supplementary Figure 6**: **XIAP confers immunity against *Shigella* infection *in vivo***

(A) Schematic presentation of the generation of conditional XIAP KO mice ($XIAP^{-/-}$; $XIAP^{\Delta hep}$). SA=splice acceptor; neo=neomycin resistance, FRT=Flp recognition target, LOX=locus of X-over P1.

(B) Wt and $XIAP^{-/-}$ mice were *i.v.* infected with invasive *Shigella* M90T or non-invasive *Shigella* BS176 with the indicated cfu. Survival was monitored for 48 h. Macroscopic inspection of representative livers ($5x10^8$ cfu, right panel). (n=5 in each group)

(C) $XIAP^{fl/fl}$ and $XIAP^{\Delta hep}$ mice were infected with *Shigella* M90T ($1x10^7$ cfu). At the indicated time points, mice were sacrificed and the liver was stained with an anti-CD45 antibody and analyzed microscopically. (scale bar = 100 µm)

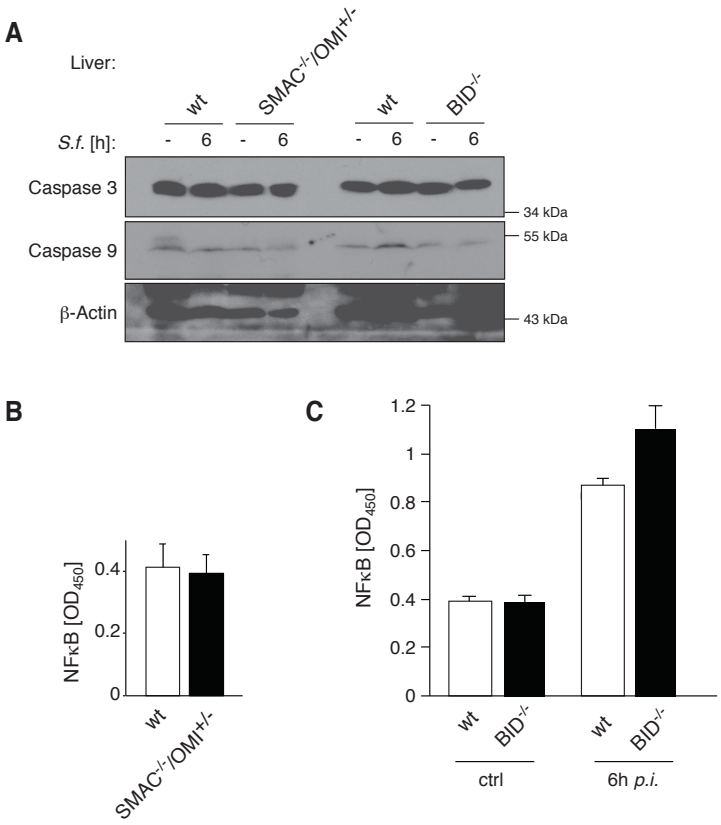
(D) $XIAP^{fl/fl}$ and $XIAP^{\Delta hep}$ mice were treated as in (B), stained with a *Shigella* specific LPS 5α-antibody and analyzed microscopically. (scale bar = 20 µm)

(E) Wt and $XIAP^{-/-}$ mice were sacrificed and liver homogenates were analyzed for basal cytokine expression using the cytokine proteome profiler array. Representative dot blot (upper panel), schematic distribution pattern of cytokines (middle panel), Quantification of dot signal intensity (lower panel).

(F) The basal level of NFκB in $XIAP^{fl/fl}$ and $XIAP^{\Delta hep}$ mice was determined in liver homogenates by ELISA.

(G) $XIAP^{fl/fl}$ and $XIAP^{\Delta hep}$ mice were left untreated (-) or were infected with *Shigella* M90T ($1x10^7$ cfu). At 6 h *p.i.* mice were sacrificed and caspase activation was determined in liver homogenates by Western blotting.

**Fig S7**




**Supplementary Figure 7: Genetic ablation of BID or SMAC restores immunity against *Shigella* infection**

(A) Wt, SMAC$^{-/-}$/OMI$^{+/-}$ and BID$^{-/-}$ mice were left untreated (-) or were infected with *Shigella* M90T (1x10$^{7}$ cfu). At 6 h *p.i.* mice were sacrificed and caspase activation was determined in liver homogenates by Western blotting.

(B) The basal level of NFκB in wt and SMAC$^{-/-}$/OMI$^{+/-}$ mice was determined in liver homogenates by ELISA.

(C) Wt and BID$^{-/-}$ mice were left untreated (-) or were infected with *Shigella* M90T (1x10$^{8}$ cfu). At 6 h *p.i.* mice were sacrificed and the level of NFκB was determined in liver homogenates by ELISA.